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Effect of Hypertrophic Calcium Signals and Altered Excitation-Contraction Coupling on the Calcineurin-NFAT Pathway
Joseph L. Greenstein, Tejas Mehta, Raimond L. Winslow.
Institute for Computational Medicine, Johns Hopkins University, Baltimore, MD, USA.
Many questions remain unanswered as to how calcium (Ca^{2+}) signals regulate the Calcineurin (CaN)- Nuclear Factor of Activated T-cells (NFAT) signaling pathway. The CaN-NFAT cascade is one of the key pathways regulating the development of cardiac hypertrophy, a condition which is recognized as a leading risk factor for heart failure. In addition, the effect of certain functional modifications in proteins involved in excitation-contraction coupling (ECC) on cardiac hypertrophy is still not well understood. In this work, we develop a mathematical model for the CaN-NFAT pathway and incorporate it into an integrative computational model of the ventricular myocyte to better understand the relationship between Ca^{2+} signals, CaN-NFAT, and hypertrophy. Model results suggest the frequency of calcium transients (i.e. heart rate) may be a primary determinant of hypertrophic signaling, whereas diastolic Ca^{2+} level and inositol trisphosphate (IP3)-mediated changes in nuclear Ca^{2+} level appear to play less of a role. Furthermore, alterations in the function of ECC proteins as may occur under conditions of stress, heart disease, and/or therapeutic intervention were investigated. Results indicate L-type Ca^{2+} channel overexpression increases NFAT activity, while overexpression of the sarcoplasmic reticulum Ca^{2+} ATPase, which mimics the functional effect of therapeutic SERCA gene transfer, has a relatively minor effect on NFAT activity. Therefore, an important prediction of this model is that enhancement of SERCA function is not predicted to promote the hypertrophic response, and it therefore may be possible to treat both pathological hypertrophy and SR dysfunction simultaneously.

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EC Coupling for Muscle Aficionados: Abnormal Contraction and Disrupted Excitability in Some Enzymatically Dissociated Skeletal Muscle Fibers
Camilo Vanegas, Martin F. Schneider, Erick O. Hernández-Ochoa.
Department of Biochemistry and Molecular Biology, University of Maryland, Baltimore, MD, USA.
Enzymatically dissociated skeletal muscle fibers are routinely used in studies of the excitation-contraction coupling (ECC) process. Here we show that skeletal muscle fibers enzymatically dissociated and cultured for 1-2 days are in general useful for experimentation; however a subset of fibers, despite their normal appearance, exhibit abnormal contractility and calcium transient alterations. We monitored the contractile response of fibers to electric field stimulation using transmitted light microscopy, and the spatial distribution of the calcium transients using Rhod-2, a non-ratiometric calcium indicator, and ultra-fast confocal microscopy. A variable fraction of fibers expressed abnormal ECC properties; they were unable to contract homogenously in response to electrical stimuli, and most notably exhibited alternating local contractions at fiber ends in response to stimuli of alternating polarity. These alternating (ALT) response fibers exhibit minor gross morphological differences from normal (WT) fibers, which contract uniformly and respond to both polarity electrical pulses. In some ALT fibers, di-8-anepps staining shows a partial disruption of the T-tubule network at the center of the fibers. This might indicate that T-tubule network disruption accompanies changes in excitability observed in ALT fibers. We hypothesize that changes in the function and/or expression of voltage gated channels, responsible for action potential generation, or voltage gated calcium channels, essential for ECC, could explain our results. Regardless of the mechanism(s) responsible for lack of excitability, we encourage the careful monitoring of the contractile response of the fiber (and if available, the evaluation of the calcium transients) to determine that normal behavior of skeletal muscle fibers be implemented when choosing fibers for physiological experiments, and recommend avoiding the use of muscle fibers that display only local contractile activity to alternating polarity. Supported by NIH-NIAMS award R37-AR055099.

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Excitation-Contraction Coupling in Human Extraocular Muscles:There is more than Meets the Eye
Marijana Sekulic-Jablanovic[1], Anja Palmowski-Wolfe[2], Francesco Zorzato[1,3], **Susan Treves**[1,4].
[1]Departments of Research and Anaesthesia, Basel University Hospital, Basel, Switzerland, [2]Eye hospital, Basel University Hospital, Basel, Switzerland, [3]Life sciences, University of Ferrara, Ferrara, Italy, [4]Life Sciences, University of Ferrara, Ferrara, Italy.
Excitation-contraction coupling is the physiological mechanism whereby an electrical signal detected by the dihydropyridine receptor, is converted into an increase in $[Ca^{2+}]$, via activation of ryanodine receptors. Mutations in RYR1, the gene encoding ryanodine receptor 1, are the underlying cause of various congenital myopathies including Central Core Disease, Multiminicore disease, some forms of Centronucleal myopathy and Congenital Fiber Type disproportion. Interestingly, patients with recessive but not dominant RYR1 mutations show a significant reduction of ryanodine receptor protein in muscle biopsies as well as ophthalmoplegia. In order to understand why the patients with recessive RYR1 mutations specifically showed involvement of the extraocular muscles, we investigated the excitation-contraction coupling machinery in biopsies obtained from ''normal'' patients undergoing resection eye surgery. Our results show that the major proteins involved in skeletal muscle excitation-contraction coupling are expressed differently in human extraocular muscles compared to leg muscles. In particular the transcripts encoding ryanodine receptor 3, cardiac calsequestrin and the alfa 1 subunit of the cardiac dihydropyridine receptor were upregulated by at least 100 fold, whereas ryanodine receptor 1 and the alfa 1 subunit of the skeletal dihydropyridine receptor were reduced 10 fold. Myotubes obtained from extraocular muscle biopsies also exhibited changes in their calcium homeostasis, and particularly the resting $[Ca^{2+}]$ was lower and the depolarization induced Ca^{2+} influx was 3 fold higher compared to that observed in leg muscle-derived myotubes. These results indicate that extraocular muscles have a different mode of calcium handling; moreover, the presence of ophthalmoplegia in patients with recessive RYR1 mutations is most likely due to the lower endogenous levels of RyR1 expressed by the extraocular muscles.

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The Calcium-Activated Chloride Channel in Zebrafish Skeletal Muscle is Activated during Excitation-Contraction Coupling
Shu Fun Josephine Ng, Anamika Dayal, Manfred Grabner.
Medical University Innsbruck, Innsbruck, Austria.
Ca^{2+}-activated Cl- channels (CaCC) are expressed in various tissues and play important roles in numerous physiological functions such as epithelial secretion, olfactory and sensory transduction, cardiac excitability, and smooth muscle contraction. Even though CaCC mRNA was identified in human skeletal muscle (Huang et al., 2006), no CaCC conductance has been reported till date. Surprisingly, we found robust Ca^{2+}-activated Cl- currents with current amplitudes of >100pA/pF at +80mV membrane potential in zebrafish skeletal muscle cells. Immunocytochemistry and subtype-specific CaCC current blockers allowed us to identify Ano1 (TMEM16A) as the channel protein responsible for this massive Cl- influx in zebrafish skeletal myotube. Whole-cell patch-clamp recordings revealed that this CaCC current is outwardly rectifying at sub-maximal Ca^{2+} levels and shows a linear current-voltage relationship at high $[Ca^{2+}]$. Interestingly, this robust CaCC current can only be observed in wild-type zebrafish myotubes, which display intact SR Ca^{2+} release during excitation-contraction (EC) coupling. In contrast, the CaV1.1 β1-null zebrafish mutant relaxed, lacking the SR Ca^{2+} release, displayed no CaCC current. Thus, the CaCC current through Ano1 is activated by SR Ca^{2+} release during EC coupling. CaCC activation during EC coupling is close to maximum regarding Cl- influx, as seen in Ca^{2+}-dependence experiments. Furthermore, we observed different CaCC current properties in the superficial slow and deep fast skeletal musculature. Further studies, to test if the CaCC current in zebrafish skeletal muscle plays a role in shaping the action potential by shortening the repolarization phase and thus allowing faster muscle contraction, are on the way.
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Muscle Regulation

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Proteins in Striated Muscles that Transcribed from the Contiguous Region of Connectin Gene
Akira Hanashima[1], Naruki Sato[2], Sumiko Kimura[2], Takashi Sakurai[1], Takashi Murayama[1].
[1]Pharmacology, Juntendo University, Tokyo, Japan, [2]Chiba University, Chiba, Japan.
Connectin is the largest protein that connects between Z-line and M-line of sarcomere and functions as a molecular spring of vertebrate striated muscles. At the contiguous region of connectin gene on mammalian genomes, there are two genes for proteins that function remain unknown. One protein (about 75kDa) consists of a SEC14 domain and three spectrin repeats, and the other protein (about 150kDa) consists of six spectrin repeats and an immunogloburin-like domain. We performed the RT-PCR experiments and revealed that these two genes are expressed in striated muscles. The western blot tests using newly produced antibodies also indicated that these proteins existed in striated muscles. The immunofluorescence microscopic observation